# "Formulation of "Novel Herbosome" of *Plumbago Indica L.* Extract and Evaluation of Its Potential in treatment of Hepatic Malignancy"

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## ➢ INTRODUCTION

Cancer, one of the most deadly challenges spreading drastically in the 21st century, has now become the most dangerous killer in the world according to the World Health Organization. [1]

Cancer is a disease characterized by uncontrolled growth and spread to other parts of the body that can result in death if not treated. Although the etiology of cancer is not completely grasped, numerous factors are known to increase risk, including many that are potentially modifiable (e.g., tobacco use and excess body weight) and inherited genetic mutations. This threat may act concurrently or in sequence to initiate and/or boost cancer growth. As per the American Cancer Society, Estimated numbers of new cancer cases and deaths in 2021 (In 2021, there will be an estimated 10.9 lakh new cancer cases diagnosed and 608,570 cancer deaths in the US.) About 608,570 Americans are expected to die of cancer in 2021, which except to about 1,670 deaths per day. Cancer is the common cause of death in the US, exceeded only by heart disease.[2]

As per National Cancer Registry Programme, India, Aizawl district about 269.4, and Papumpare district about 219.8 had the highest age-adjusted frequency among males and females. The estimated number of patients with cancer in India is 1,392,179 for the year 2020. [3]

Ayurvedic medicines world's oldest Indian Medical Systems for preventing or vanquishing various tumors using herbal extracts and nowadays scientists are keener to research harmonious and alternative medicine for the control of cancer. In the Ayurvedic conception, reported to 'Charaka' and 'Sushruta Samhitas' cancer is described as inflammatory or non-inflammatory swelling and mentioned either as 'Granthi' (minor or 'Arbuda' (major). [4] [5]

Vata or air, Pitta or fire, and Kapha or water are three basics of Ayurveda and are very major for normal body function. In malignant tumors, all three systems get out of control (Tridoshas) and lose mutual kinship that etiology tissue damage, resulting in evaluative conditions. Tridoshas cause excessive metabolic crisis resulting in augmentation.[6]

Herbosomes are a novel idea of Herbo-phospholipid composition for herbal drug delivery. Herbosomes is a herbal drug technology that removes the limitations of the indigenous drug delivery systems. Plant-based herbal formulations have been widely consumed all over the world by patients for their better therapeutic effects as they have no or fewer side effects as compared to modern medicines. Herbosome structures contain the active drug of the herb bounded with water-soluble as well as a fat-soluble phospholipid, because of paired solubility herbal formulation that is better absorbed, enhanced bioavailability such as Ginkgo biloba, milk thistle, grape seed, ginseng, and the like. [7]

Benefits over traditional drug delivery systems

1. Herbosome shows better stability
2. It enhances the absorption of herbal constituents
3. Drug dose reduced
4. More stable in gastric secretion and resist the action of gut bacteria
5. Enhanced permeability [8]

## ➢ HYPOTHESIS

The plant is a source of primary metabolites such as amino acids, fats, sugar along with secondary metabolites like alkaloids, flavonoids, glycosides, terpenes, and others. which shows pharmacological/therapeutic actions but sometimes absorption is poor because of size is large or small, poor solubility, which affects on bioavailability of the drug.

The therapeutic actions of herbal drugs depend on the drug delivery system and effective level of the active compound.

To improve the bioavailability and overcome these barriers for better therapeutic effects; the active chemical constituents can be converted into lipid compatible molecular with phospholipids complex.

Based on this hypothesis, Herbosome manufactured by using active chemical constituents of plants and phospholipids will show better bioavailability and lesser adverse drug reactions.

## ➢ REVIEW OF LITERATURE

### ➢ Anti-fungal activity

Methanol extract showed strong antifungal activity with zone inhibition of (10.0-27.0) mm while the highest antifungal activity was seen against *C. albicans, B. lastomyces dermatitides,* Microsporum spp, and *Trichophyton spp*. *C. albicans* the more susceptible fungal strain of the methanolic extract of *Plumbago indica* L. [9]

Ethanolic extract of *Plumbago indica* roots showed antifungal activity against *Aspergillus niger* and *C. albicans***.**[10]

Plumbagin was reported as the active constituents against *C. albicans* with MIC and MFC values 0.78 and 1.56µg/mL.[11]

### ➢ Anti-Acne Activity

The MIC of the acetonic extracts of *Plumbago indica* roots against *P. acnes, S.epidermidis,* and *M. furfur* was found to be 600, 200, and 300µg/mL.[12]

The anti-acne activity of acetone extract of *Plumbago indica* (in gel formulation) was investigated against *P.acnes, S.epidermidis,* and yeast by well diffusion method has potential activity against acne-causing microorganisms.[13]

### ➢ Anti-Microbial activity

Methanolic extract of *Plumbago indica* was showed antibacterial activity with zone inhibition ranging 7.0-25.0mm against *Staphylococcus aureus, Salmonella typhi,* and *Salmonella paratyphi* with MIC was found 31.25-125µg/mL with the use of ciprofloxacin as a standard.[14]

PPE (Plumbago derivative-rich *Plumbago indica* root extract) was not less than 13.0%w/w contains and shows antibacterial activity against *Propionibacterium acnes, Staphylococcus aureus,* and *Staphylococcus epidermidis* by using the micro dilution assay method, and its stability was determined by a period of four months stored as a dried powder in a well-closed container protected from light under 4±2°C.[15]

A disc diffusion assay of Plumbago revealed potent antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. Molecular docking of Plumbago revealed high specificity towards the DNA *gyrase* binding site with a high fitness score and a minimum energy barrier of - 7.651kcal/mol.[16]

- **Anti-fertility Activity**

  *Plumbago indica* showed a percentage pre-implantation loss of 40% and 50% against control at doses of 200 and 400mg/kg b/w. *Plumbago indica* and *A. lanata* at a focusing of 10% have shown no motility. Both *Plumbago indica* and *A. lanata* at a concentration of 10% have shown no motility of rat spermatozoa within 60sec.[17]

  Acetone extracts of *Plumbago indica* stem possess activity in female albino rats at doses 200 and 400mg/kg. The anti-ovulatory activity was reversible when discontinuing extract and showed significant estrogenic and anti-estrogenic activity.[18]

- **Analgesic Activity**

  Methanolic extract of *Plumbago indica* has been shown significant inhibition of pain response by using the acetic acid-induced writhing method at doses 250mg/kg and 500mg/kg in young swiss-albino mice.[19]

  Aqueous extracts of *Plumbago indica* was estimate with a dose 300mg/kg body weight; carrageenan-induced paw volume show analgesia 68.29% and 45.2% determined by Eddy's hot plate method. [20]

- **Anti-Malarial activity**

  In vitro antimalarial activity of Plumbago was investigated in *Plasmodium* berghei-infected mouse model at the dose of 25mg/kg body weight given for 4 days was safe and produced antimalarial activity.[21]

- **Anti-bacterial activity**

  MIC of methanolic extract was found to be 20µg/mL on both *E. coli* and *K. pneumonia*, while aqueous extract had a Microbial Inhibitory concentration of 10 and 20µg/mL of *E. coli* and *K. pneumonia*.[22]

  Ethanolic extracts (80%) posses activity against *B.subtilis, S.aureus, E.Coli, P.aeruginosa*, MIC is 6.25mg/mL for *B.subtilis* and 12.5mg/mL for *S.aureus, E.Coli, P.aeruginosa*.[23]

  Plumbagin was exhibited activity against *S. aureus* with MIC and MBC/MFC value 1.56 and 25.0µg/mL.[24]

- **Anti-mycobacterial activity**

  Using a purified preparation of the recombinant version of MtbThyX, a structurally related compound inhibits its activity leads to cell dying. Plumbago quashes mycobacterial cells primarily by targeting ThyX, a vital enzyme required for their abidance.[25]

- **Antioxidant Activity**

  In vitro antioxidant activity of methanolic extracts of *Plumbago indica* by using 2,2-diphenyl-1-picrylhydrazyl in an assay based on the method Oyaizu along with Hydroxyl radical scavenging activity. [26]

- **Hepatoprotective Activity**

  The ethanolic root extract of *Plumbago indica* was afforded significant protection against paracetamol-induced hepatocellular injury treated with 200mg/kg and 400mg/kg of body weight with reference drug-Silymarin (100mg/kg).[27]

- **Anti-influenza Activity**

  Methanolic and ethanolic extracts of *Plumbago indica* exhibited 100% reduction in both simultaneous and post treatment assay at concentration 10mg/mL, 5mg/mL and 1mg/mL[28]

- **Cardio Protective**

  After Plumbagin treatment of animals, regained the body weight and heart weight and ameliorated by plumbagin treatment, from doxorubicin-induced damage.[29]

  Plumbagin increased activities of creatine kinase and cardiac troponin and restored the level of lipid peroxide markers, increased antioxidative enzymes, decreased pro-inflammatory cytokines by oral administration. [30]

  Oxidative stress was overcome by plumbagin by decreasing ROS and lipid peroxide levels in animals with myocardial injuries, with redox imbalance by injury by transcription factors NF-kB and Nfr-2. Pro-inflammatory cytokine expressions were significantly revoked by plumbagin treatment. [31]

- **Macrofilaricidal Property**

  That property Plumbago indica was reported against Setariadigitata, an ailarial parasite of cattle with an inhibitory concentration range from 0.02 and 0.05mg/mL.[32]

- **Anti-fertility Activity**

  Acetonic and Ethanolic extracts exhibit antifertility activity with doses 200 and 400mg/kg measured on estrogenic activity in rats and confirmed by histopathology study of the uterus.[33]

  Ethanolic extract of *Plumbago indica* roots shows the anti-implantation effect with loss of 40% and 50% against control at dose 200 and 400mg/kg b/w which recommended percentage defeat was 30% and 40% at 200 and 400mg/kg b/w. [34]

Plumbagin (acetone extract) with the dose levels, 200 and 400mg/kg body weight showed significant estrogenic and anti-estrogenic activity ($p< 0.05$) ($p< 0.001$) in female rats. [35]

- **Anti-Inflammatory Activity**

  Plumbagin suppresses T cell proliferation in response to polyclonal mitogen Concanavalin A by blocking cell cycle progression induced IL-2, IL-4, IL-6, and IFN-γ cytokines. Also suppressed CD69 and CD25 in activated T cells.[36]

- **Genotoxic Effect**

  Ethanolic extract of Plumbago indica root (EEPIR) serves potential therapeutic effects at concentration 25-100µg/mL induced cell kills and cytotoxic effect at concentrations of ≥500µg/mL in vitro.[37**]**

- **Anti-Cancer Activity**

  Methanolic extract of *Plumbago indica L.,* against brine shrimp nauplii and evaluate LC50 and LC90 was 5.0µg/mL and 12µg/mL.[38]

  Ethanolic extract (50-250µg/mL) reflects cytotoxicity in HE-17 cell lines by using MTT assay.[39]

  Plumbagin down the viability of human prostate cancer cells (PC-3, LNCaP, and C4-2) with ROS generation and depletion of intracellular GSH levels including superoxide dismutase 2 (Mn-SOD).[40]

  Plumbagin acted as a potent inducer of ROS, suppression of cellular glutathione, proteasome inhibitor causing DNA double-strand break by oxidative DNA base damage. [41]

  Plumbagin inhibits tumor development and no sign of toxicity at 200 or 500ppm dose levels decrease PKCE, AKT, Stat3, and COX2 compared with control group both primary and castration-resistant prostate cancer in Pten-KO mice.[42]

- **Anti-proliferative effect**

  *Plumbago indica* L., *P. zeylanica L,* in stomach and breast cancer cell lines, confirmed cell kills by nucleus staining. [43]

- **Anti-Diarrhoeal Effect**

  Plumbagin produce delayed intestinal motility and inhibit smooth muscle contractility; inhibits both Ca2+ and cAMP-activated Cl-channels as per mechanism and can be used in the treatment of CT-induced, Travelers and rotaviral diarrhea with the inhibition of CaCC–calcium-activated chloride channel and cystic fibrosis trans membrane conductance regulator (CFTR) in both HT-29 cells and mouse colon. [44]

- **Toxicity study**

  *Plumbago indica* root crude extract in hamsters mortality, behavior, histochemical, histopathological replaces with dose at 0, 100, 400, 1000, and 3000 mg/kg orally for 24h and every day for 7 days assessment. The parameters show differences in liver function tests (ALT and AST) and kidney function tests (creatinine and BUN). [45]

## ➢ AIM AND OBJECTIVE

In modern medical science, the symptoms frequently appear in various diseases or disorders as a result of different types of deficiencies nowadays related to modern medical therapies includes toxicity, ADR, etc., and several health issues that will generate toxic effects.

Due to these weaknesses/defects of modern allopathic therapy, the way to increase towards herbal drugs.

The current research work includes identification, extraction, isolation, characterization, formulation, and pharmacological evaluation of liver cancer potential on *Plumbago indica L.*

**1.** To study Plant material for identification and authentication.

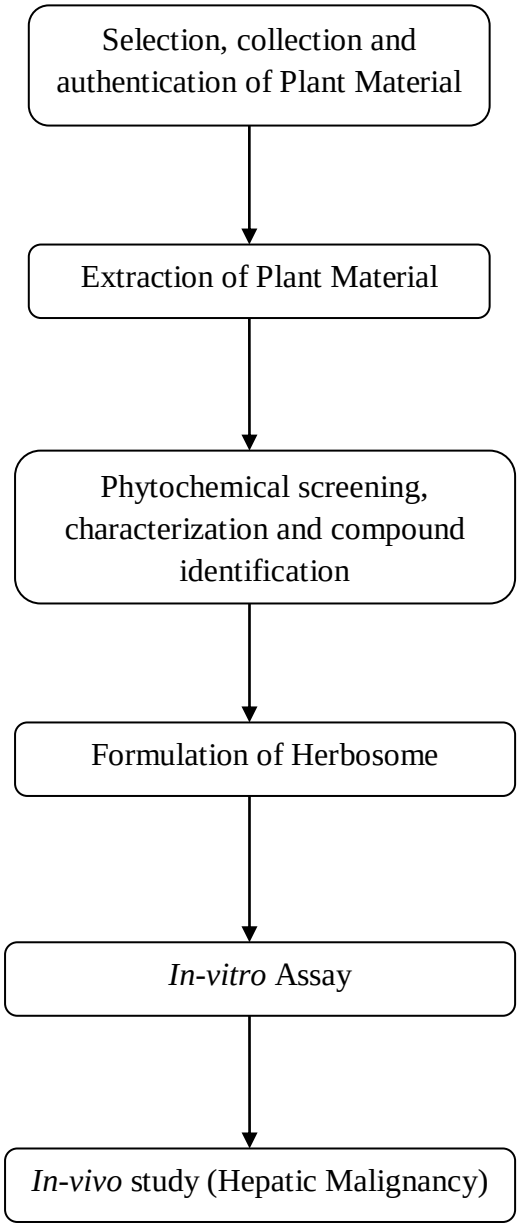
**2.** To study phytochemical screening and characterization of novel compounds.

**3.** To perform formulation of Herbosome.

**4.** To study in-vitro assay of formulation using cancer cell lines.

**5.** To study in-vivo study – DENA-induced Liver cancer.

## ➢ METHODOLOGY


Selection, collection and authentication of Plant Material
Extraction of Plant Material
Phytochemical screening, characterization and compound identification
Formulation of Herbosome
*In-vitro* Assay
*In-vivo* study (Hepatic Malignancy)

- The root part of *Plumbago Indica L* will procure and collect and identification of the plant will be done through available literature and authentication will be carried out.
- *Plumbago Indica L* roots will dry. The fine crude powder will use as a herbal drug. Weight amount of dried powdered *Plumbago Indica L* roots will extract in different solvents by extraction methods.
- The preliminary phytochemical investigation will be carried out using well-established methods.
- Formulation of Herbosome will be prepared with phospholipid and polymers based on compatibility and interaction of drug.
- The following methods will try in trial batches for preparing Herbosome of *Plumbago Indica L* root extract
    - Anti-solvent precipitation method
    - Solvent evaporation method
    - Film hydration technique
    - Anhydrous Co-solvent Lyophilization method
- Evaluation of Preliminary batches will be carried out by shape, percentage of yield, particle size, zeta potential, drug entrapment, etc.
- *In-vitro assay* – Hep G2 cell lines will obtain and will be used to study cytotoxicity by MTT viability assay.
- The safety of formulation of plant extract will be carried out by acute oral toxicity study
    - The acute toxicity of the plant material will be evaluated as per OECD guidelines 423, Four groups, with animals; will receive a dose of 5mg/kg, 50mg/kg, 300mg/kg & 2000mg/kg body weight. Animals will observe daily for 14 days in an animal house approved by the institutional animal ethics committee.

- *In-vivo assay* anticancer activity will be carried out by DENA induced hepatic malignancy
    - The animals will be divided into the following groups (n=6).

      Group I: Control

      Group II: DENA induced Liver cancer

      Group III: Plant Extract at low concentration

      Group IV: Plant Extract at High concentration

      Group V: Isolated compound

      Group VI: Herbosome formulation
- Parameters such as tumor diameter, tumor weight, tumor yield, SGOT, SGPT, ALP, Bilirubin, Lipid peroxidation, SOD, will be carried out along with histopathology of the liver also carried.
- Data will be tabulated interpretation, analyzed statically and presentation.

**Importance/Society application**

The utilization of herbs as a drug has a long history throughout the world and herbal formulations also are found in Pharmacopeia.

In recent years especially in Corona Pandemic, there has been more interest developed/generated for herbal formulations throughout in the world, because modern medicine is no cure-all solution against disease or disorders, unwanted side effects.

With the use of herbal formulation, any kind of disease/disorder can cure therapeutically without showing toxic effects.

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